Production and Evaluation of Papain and Pectinesterase Enzymes from Papaya Fruits

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Abstract

The objective of this study was to produce and evaluatethe papain and Pectinesterase enzymes from papaya (Carica papaya L.) fruit. The protein content and proteolytic activity were found to be 0.74mg/ml and 7.68 U/ml of papain enzyme, 0.48mg/ml and 2.8 U/ml of papaya pectinesterase, respectively. The optimum NaCl concentration on PE activity was found to be 0.3M. The optimum pH on the reaction activity of crude papain and papaya pectinesterase were found to be 6.4 and 7.0, respectively.The optimum temperature was found to be 60ºC and 55ºC, respectively.The optimum enzyme concentration was found to be 0.6g/100ml and 12ml of the extract, respectively. The optimum substrate concentration was found to be 1.1g/100ml and 6.5 mg/ml.TheMichaelis –Menten constant and maximal rate of the crude papain and papaya Pectinesterase equaled to 0.43 g/100ml potassium phosphate buffer solution and 13.68 U/ml for the first enzyme while, these values were 3.2 mg/ml and 15.6 U/ml for the second ones, respectively.

Keywords: Papain, Pectinesterase, Papaya fruits, Proteolytic activity, Kinetic parameters.

Introduction

Papaya fruit *(Carica papaya L.)* or the pawpawis a popular and economically important fruit tree of tropical and subtropical countries**.**The fruit is consumedworld-wide as fresh fruit and as a vegetable or used as processed products**(Teixeira da Silva *et al.,* 2007).** Its high content of vitamin C, E and provitamin A, which has a protective effect against cancers, so adding a daily serving of papaya to diets may lessen the risk of developing cancer. Also, rich in fiber, which travels through the body and binds itself to cancer-causing toxins in the colon and its low calorie status that is recommended for low hypo caloric diets,**Pamela and James (2010).**

The papain (E.C. 3.4.22.2.) is a natural proteolytic enzyme that was extracted from the latex of papaya's unripe fruits **(Baeza*et al.,* 1989)**that digest protein and used as meat tenderizer, as digestive medicine, in pharmaceutical, brewing and tanning industries, and in manufacturing of chewing gum, **Puig*et al.* (2008).**

Rao*and Rangan* (2001)studied the production of crude papain from papaya latex and by-product. Latex containing papain was extracted from 70-to 100-day-old fruits of pawpaw by tapping 4 or 6 times on the fruit. Latex was subsequently collected from the same fruit at 4-and 7-day intervals.**Dayanand (2013)** studied the evaluation of comparative total proteolytic activity in plant lattices. He found that the total proteolytic activity of *Carica papaya* latex (crude papain) solubilized in water at pH 7.6 and 37ºC was 1.750 units, 128 mg total protein, 13.6 (activity/mg protein) specific activity. Also, he evaluated the total proteolytic activity at different pH conditions and the specific activity by using protein as substrate, respectively.Pectinestarase (PE, pectin methylesterase, pectin pectylhydrolase, EC, 3.1.1.11) removes methoxyl groups from methylated galacturonic residues of pectic substances, **(Vanden broeck*etal.,* 1999).** This enzyme is widely distributed in higher plants and can be found in different plant tissues; mainly those contained in fruits **(Baron and Thibault, 1985).** Pectinesterase play a central role in the process of fruit softening during ripening, in addition influences the quality and stability of processed products. A short period after papaya is pulped into puree, a gel is formed. This gel formation has been attributed to the enzymatic action of pectinesterase. Also, of great concern to the citrus industry since it has been definitely established that it is the causative agent for clarification of citrus juices and gelation of concentrates,**Sarr and Tsai (2008),**the enzyme must be inhibited by pasteurization at90 ^{0}C for one minute.

The aim of this investigation is to study the production and evaluation of papainand Pectinesterase enzymesfrompapaya fruits and kinetic parameters of these enzymes.

Materials and Methods

Papaya (*Carica papaya L.)* fruits solo variety was obtained from farm agricultural research station, Faculty of agriculture at Moshtohor, BenhaUniversity. Samples were collected in 2012 and 2013.

Preparation of papaya fruit pulp:-

The fruits were used at two maturity stages, mature green and ripe stages (100% yellow). Ripe fruits were washed and left to drain. Then manually were peeled, deseeded and then were cut to small pieces and converted into smooth papaya pulp using mixer or blender (model–brown). Samples from the fresh pulp were used to the evaluation of pectinesterase (PE). The remaining pulp was stored in containers at the deep freezer for further processing. Papaya latex used in this work was collected from unripe papaya

fruits to study the kinetics parameters of produced papain enzyme.

Extraction of crude papain (papaya latex protease)and papaya pectinesterase enzyme (PE).
The extraction of papaya latex protease was carried out according to the method described by **(Nitsawang, *et al.* 2006).**Also, the extraction of papaya Pectinesterase was performed according to the method described by **Wicker (1992).**

Effect of NaCl on PE extraction and activityfrom papaya fruits:-
The effect of NaCl concentrations (0.5 – 3.0 M) on the extraction of papaya pectinesterase was studied.Also, the effect of NaCl concentration on PE activities was tested in the range (0.05 – 0.5 M) NaCl in the reaction mixture. Measurements were carried out by using 1% pectin dissolved in NaCl solution of varying concentrations at pH 7.0 and at 30ºC according to the method of **Wieker (1992).**

Proteolytic activity and pectinesterase activity:-
Proteolytic activity was determined according to the method described by **Arnon, (1970).** Pectinesterase (PE) activity was assayed by the titration method proposed by **Kertesz (1955).**Protein enzyme content was determined according to the method described by **Bradford(1976)**, using bovine serum albumin (BSA) as standard.

Kinetic parameters of crude papain and papaya pectinesterase enzymes:-
The effect of pH on the crude papain enzyme activity was measured at different pH values ranging from (5.8 – 8.0) by using 0.05 M phosphate buffer.Also, the effect of pH on the papaya pectinesterase enzyme activity was determined at different pH values ranging from (5.0- 9.0) by using 10 ml of 1% pectin solution containing 0.15 MNaCl solution. All reactions were carried out for 10 min at different pH values and at 30ºC.The activities of crude papain and papaya pectinesterase were measured at different degrees of temperatures ranging from 30 – 80ºC.The effect of enzyme concentration on crude papain activity was measured at different enzyme concentrations values ranging from (0.1- 1 %).However, the activity of papaya (PE) was measured by using different volumes of enzyme extract solution. A valid assay was established by using different enzyme concentrations (1-10 ml) and 10 ml of 1% pectin in 0.15M NaCl solution, at optimum pH and temperature. Solutions of casein varying in concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4 and 1.5%, w/v) were employed to study the effect of substrate concentration on crude papain activity at optimum pH, temperature and constant amount of enzyme concentration according to the methods of **(Arnon, 1970).**Also, solutions of pectin varying in concentrations (0.5, 1.0, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 and 10 mg/ml) were employed to study the effect of substrate concentration on pectinesterase activity at optimum pH, temperature and constant amount of pectinesterase enzyme according to the methods of **Fayyaz*et al.* (1995).**Michaleis constant (K_m) and maximum reaction velocity (V_{max}) were calculated, then **Lineweaver and Burk (1954)** plots were determined**.**

Results and Discussion

Extraction of papain and papaya pectinesterase from papaya fruits:-
The amount of fresh latex obtained by the first fruit tapping was 6.0 g/kg fruit, and after the drying process the amount of crude papain obtained was 1.52 g/kg fruit, the moisture content was found to be 74.6% of papaya latex protease. Also, the effect of sodium chloride concentrations (0.5- 3 M) on the extraction of papaya pectinesterase (PE) is presented in Fig (1). Raising the NaCl concentration in the extracting solution from (0.5 to 1.0 M) increases the activity of PE, reaching its maximum activity (6.25 units /mg protein) at 1.5 M of NaCl. This concentration of NaCl for extracting PE for papaya fruits was similar to value obtained by **Fayyaz and Asbi (1993),** they found 1.7M the optimum concentration of PE extracting from guava, and also it is lower than reported by **Maria *et al.* (2007),**who found that 2 Mis the optimum NaCl concentration for hawthorn PE extraction. The strong influence of NaCl on papaya PE extraction suggested that the enzyme associated with the cell wall by ionic interaction. The decrease in PE activity with NaCl concentrations higher than 1.5 M might arise as an effect of salting out **(Polacsek-Ra´cz and Posar-Hajnal, 1976).** These variation between the obtained and other results may be due to the different sources produced this enzymes.

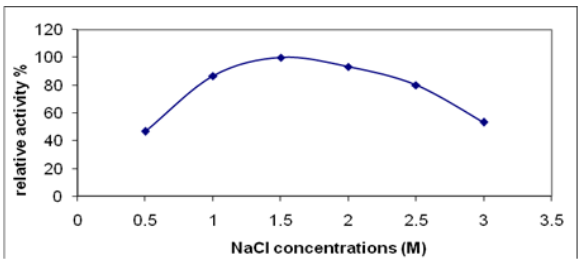


Fig. (1). Effect of NaCl concentrations on papaya pectinesterase extraction.

Factors affecting on the reaction velocity and activity of crude papain and pectinesterase enzymes:-
The proteolytic activity and protein content of crude papain enzyme was determined and the obtained results are illustrated in Table (1). From these data

the activity was found to be 7.68 U/ml, 0.74 mg/ml of protein content and 10.38 U/mg protein of specific activity. The obtained results are in agreement with that stated by **Rofael and Salwa (2003).**On the other hand, the activity and protein content of pectinesterase enzyme was determined and the results are tabulated in Table (1). From these data the activity was found to be 2.8 micro-equivalent COOH/min/ml crude extract, 0.48 mg/ml of protein content and 5.83 units/mg protein of specific activity the obtained results are in agreement with that stated by **Fayyaz*et al.* (1995).**

Table 1. Activity and protein content of crude papain and papaya Pectinesterase enzymes.

Extracted Enzymes	Obtainedof tyrosine equivalents (µm/ml)	Activity (U/ml)	Protein content (mg/ml)	Specific activity (U/mg protein)
Crude papain	0.64	7.68	0.74	10.38
papaya pectinesterase	----------	2.8	0.48	5.83

Effect of NaCl concentrations on the reaction activity of papaya pectinesterase (PE) enzyme:-
The effect of NaCl concentration on PE activity was tested in the range (0.05- 0.5 M) NaCl in the reaction mixture. Measurements were carried by using 1% pectin, pH 7.0 at 30ºC.
The accomplished results are shown in Table(2) and Fig (2). The obtained result indicates that papaya pectinesterase activity like other plant pectinesterase also depended on the NaCl concentration in the assay. The effectiveness of the salt was related to its concentration in the assay mixture and the activity increased with the concentration up to 0.3 M, but on raising NaCl level further the activity decreased. This optimum level of NaCl concentration obtained in the present study is lower than that observed by **Maria *et al.* (2007)** and is higher to the value of (0.25M) NaCl reported by **Fayyaz*et al.* (1995).**

Table 2. Effect of NaCl concentration on the reaction activity of papaya pectinesterase (PE) enzyme.

NaCl Conc. (M)	Activity (units/ml)	Specific activity U/mg protein	Relative activity (%)
0.05	**3.6**	**7.50**	**23.07**
0.10	**6.6**	**13.75**	**42.30**
0.15	**8.4**	**17.50**	**53.85**
0.20	**11.4**	**23.75**	**73.07**
0.25	**13.8**	**28.75**	**88.46**
0.30	**15.6**	**32.50**	**100.00**
0.35	**15.0**	**31.25**	**96.15**
0.40	**12.0**	**25.00**	**76.92**
0.45	**7.8**	**16.25**	**50.00**
0.50	**7.2**	**15.00**	**46.15**

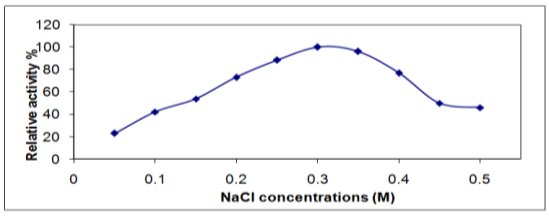


Fig (2). Effect of NaCl concentration on the reaction activity of papaya pectinesterase (PE) enzyme.

Effect of pH on the reaction activity of crude papainand papaya pectinesterase (PE) enzymes:-
The effect of pH on the reaction activity of crude papain was evaluated at different pH values. The reaction mixtures were prepared in different solutions at pH values of 5.8, 6.0, 6.2, 6.4, 6.6, 6.8, 7.0, 7.2, 7.4, 7.6, 7.8 and 8.0 with 0.65% (w/v) casein by using potassium phosphate buffer at pH 7.5 and 37°C.The enzyme activities obtained are presented in Table (3) and Fig. (3) The maximum enzyme activity i.e. 8.16units/ml was observed at pH 6.4 for soluble crude papain enzyme. The bell-shaped curve (Fig., 3) was obtained with a relatively plateaus and with sharply decreasing rate on the right side. The present papain pH optimum of pH 6.4 is lower than the pH optima of (7.0) reported forthe optimum conditions for the activities of both crude extracted enzyme and commercial papain,**Pitpreecha and Damrongsakkul (2006).**

On the other hand, the obtained results for papaya pectinesterase activity as a function of pH is shown in Table (4) and illustrated in Fig (4) The obtained results, indicated that the activity gave pH optima of 7.0 The present PE pH optima of 7.0 was slightly higher than the pH optima of 6.5 reported bypectinesterase from plant tomato,**Nikolic and Mojovic (2007)**. This value differed from that

reported by**Denes *et al.* (2000)** and **Fayyaz*et al.* (1995)**.

Table3. Effect of pH on the reaction activity of crude papain enzyme**.**

pH's	Obtained of tyrosine equivalents (µm/ml)	Activity (units/ml)	Specific activity U/mg	Relative activity (%)
5.8	**0.62**	**7.44**	**10.05**	**91.17**
6	**0.63**	**7.56**	**10.21**	**92.64**
6.2	**0.64**	**7.68**	**10.37**	**94.11**
6.4	**0.68**	**8.16**	**11.03**	**100.00**
6.6	**0.62**	**7.44**	**10.05**	**91.17**
6.8	**0.61**	**7.32**	**9.89**	**89.70**
7.2	**0.60**	**7.2**	**9.72**	**88.23**
7.4	**0.58**	**6.96**	**9.40**	**85.29**

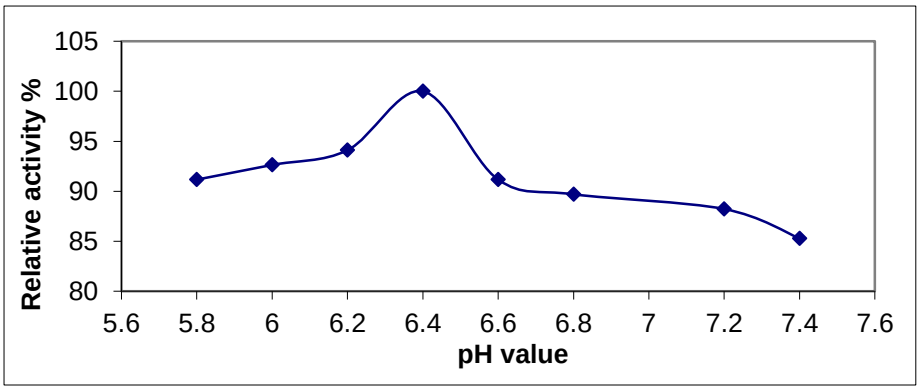


Fig (3): Effect of pH on the reaction activity of crude papain enzyme.

Table 4. Effect of pH on the reaction activity of crude papaya pectinesterase (PE).

pH's	Activity (units/ml)	Specific activity U/mg protein	Relative activity (%)
5.0	**5.4**	**11.25**	**31.03**
5.5	**7.8**	**16.25**	**44.83**
6.0	**10.8**	**22.5**	**62.06**
6.5	**14.4**	**30.0**	**82.76**
7.0	**17.4**	**36.25**	**100.00**
7.5	**16.2**	**33.75**	**93.10**
8.0	**14.4**	**30.0**	**82.75**
8.5	**10.8**	**22.5**	**62.07**
9.0	**6.0**	**12.5**	**34.48**

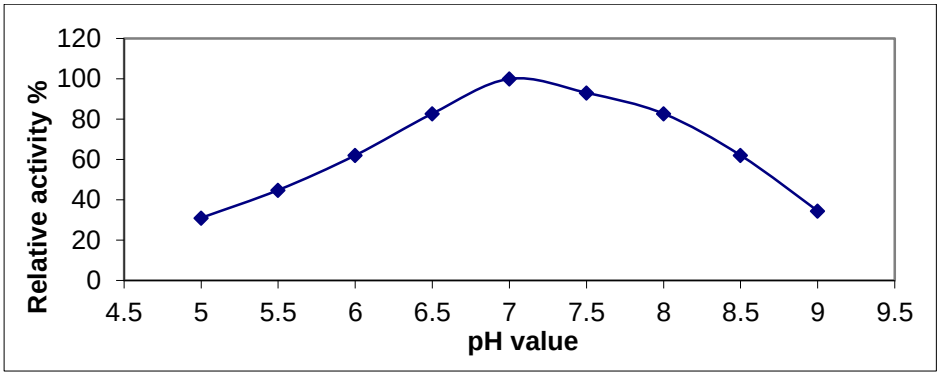


Fig (4): Effect of pH on the reaction activity of crude papaya pectinesterase (PE) enzyme.

Effect of temperature on the reaction activity of crude papain and papaya pectinesterase (PE) enzymes:-

Eleven different temperatures, i.e. 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 and 80°C were chosen to investigate the optimum of crude papain enzyme. The experiments were carried out at optimum pH and the standard assay conditions. The obtained results are tabulated in Table (5) and illustrated in Fig. (5).

Maximum activity of crude papain was obtained at 60°C. The maximum rate of reaction for crude papain enzyme was found to be 8.64units/ml. Such values for optimum temperature and maximum activity are different with these previous study in which noted the optimal temperature of papain were 65°C, **Kilinc*et al.* (2001),**and 70°C,**Roy *et al.* (2005).**

Table5. Effect of temperature on the reaction activity of crude papain enzyme.

Temperature (ºC)	Obtained of tyrosine equivalents (µm/ml)	Activity (units/ml)	Specific activity U/mg protein	Relative activity (%)
30	**0.38**	**4.56**	**6.16**	**52.77**
35	**0.52**	**6.24**	**8.43**	**72.22**
40	**0.54**	**6.48**	**8.75**	**75.00**
45	**0.49**	**5.88**	**7.94**	**68.05**
50	**0.51**	**6.12**	**8.27**	**73.44**
55	**0.69**	**8.28**	**11.19**	**95.83**
60	**0.72**	**8.64**	**11.68**	**100.00**
65	**0.66**	**7.92**	**10.70**	**95.04**
70	**0.65**	**7.80**	**10.54**	**93.60**
75	**0.60**	**7.20**	**9.72**	**86.40**
80	**0.33**	**4.00**	**5.41**	**46.20**

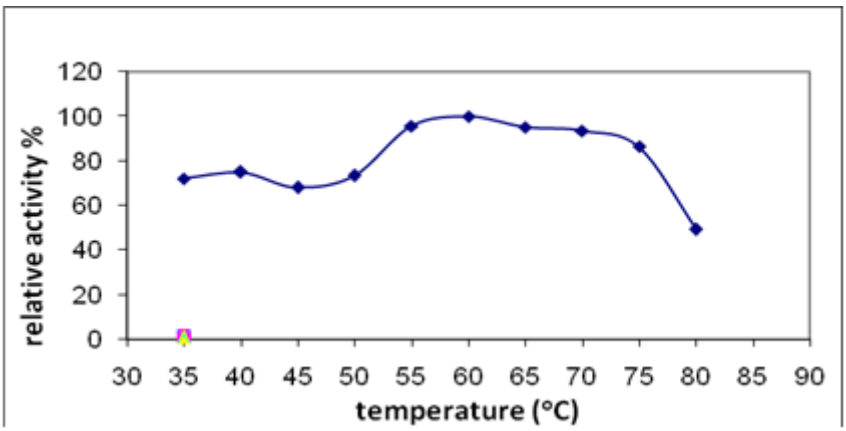


Fig (5): Effect of temperature on the reaction activity of crude papain enzyme.

Table 6. Effect of temperature on the reaction activity of crude papaya pectinesterase (PE) enzyme :-

Temperature (ºC)	Activity (units/ml)	Specific activity U/mg protein	Relative activity (%)
30	**9.0**	**18.75**	**45.50**
35	**10.8**	**22.50**	**54.50**
40	**13.2**	**27.50**	**66.60**
45	**15.6**	**32.50**	**78.80**
50	**17.4**	**36.25**	**87.90**
55	**19.8**	**41.25**	**100.00**
60	**18.6**	**38.75**	**93.90**
65	**18.0**	**37.50**	**90.91**
70	**16.8**	**35.00**	**84.85**
75	**12.0**	**25.00**	**60.61**
80	**10.2**	**21.25**	**51.52**

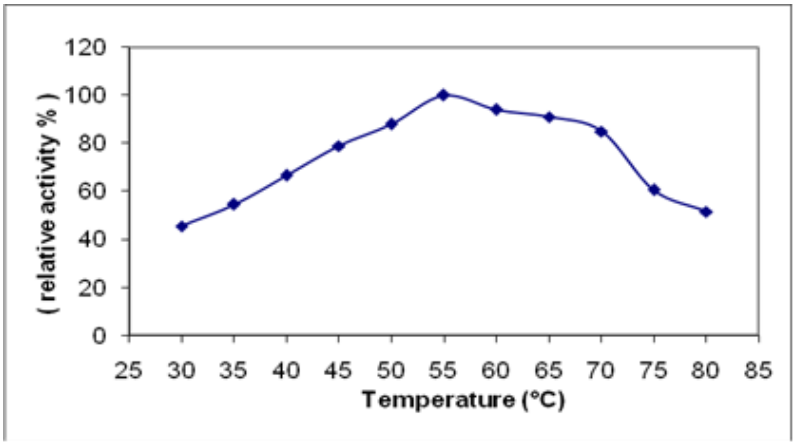


Fig (6): Effect of temperature on the reaction activity of crude papaya pectinesterase (PE) enzyme.

However, Papaya pectinesterase showed an optimum reaction temperature at 55^{0}C Table (6) and illustrated in Fig (6). Over the temperature range (30- 80^{o}C) was studied, the rate of deesterification was increased from 30^o to 55^{o}C until reached its maximum at 55^{o}C and decreased after 70^{o}C.The obtained optimum temperature was typical as found by **Maria *et al.* (2007)** who found 55^{o}C to be the optimum temperature for pectinesterase from Hawthorn (*crataeguspubescens*) fruit. Also, it was close to those reported for the PE from apple by **Denes *et al.* (2000)**; bramley apple PE **King (1990)**; orange PE by **Korner*et al.* (1980)** and potato PE **Saenz *et al.* (2000)**. Those authors reported that 60^{o}C was the optimum temperature for pectinesterase activities.

Effect of enzyme concentration on the reaction activity of crude papain and papaya pectinesterase (PE) enzymes:-

The effect of crude papain enzyme concentrations on the rate of enzymic hydrolysis of casein are presented in Table (7) and illustrated in Fig. (7).Ten differents enzyme concentrations i.e. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0% (w/v) were tested for the optimal enzyme concentration at pH 6.4, 60°C and the incubation time was 10 min. The obtained results showed that the maximum activity reached to 9.6Units/ml at enzyme concentration of 0.6% (v/v). On the other hand, the reaction activity was decreased by increasing the amount of enzyme added. These light decreases might be attributed to the rate of the reverse reaction or inhibition effect which might be processed in opposite direction and steric hindrance is originated from the excess amount of enzyme. The obtained results are in agreement with that obtained by**Kilinc*et al.* (2001) and Roy *et al.* (2005)**.

While, the effect of different enzyme concentrations on the activity of crude papaya pectinesterase was evaluated. Different enzyme amounts i.e. 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 13.0, 14.0 and 15.0 ml were tested. The reaction activity of PE increased as the volume of enzyme concentration used increased till the volume of 12 ml as shown in Table (8) and illusterated in Fig (8). Then there was no further increment of activity as more enzymes were used. These observations may be attributed to the rate of the reverse reaction (inhibition effect) of the product which might produced in opposite direction leading to general decrease in the final product, **Illanes*et al.* (1998).**

Table 7. Effect of enzyme concentration on the reaction activity of crude papain enzyme.

Enzyme conc. (%)	Obtained of tyrosine equivalents (µm/ml)	Activity (units/ml)	Specific activity U/mg protein	Relative activity (%)
0.1	**0.52**	**6.24**	**8.43**	**65.00**
0.2	**0.60**	**7.2**	**9.72**	**75.00**
0.3	**0.64**	**7.68**	**10.37**	**80.00**
0.4	**0.65**	**8.2**	**11.08**	**85.41**
0.5	**0.70**	**8.40**	**11.35**	**87.5**
0.6	**0.80**	**9.60**	**12.97**	**100.00**
0.7	**0.74**	**8.88**	**12.00**	**92.50**
0.8	**0.73**	**8.76**	**11.83**	**91.25**
0.9	**0.70**	**8.40**	**11.35**	**87.50**
1.0	**0.70**	**8.40**	**11.35**	**87.50**

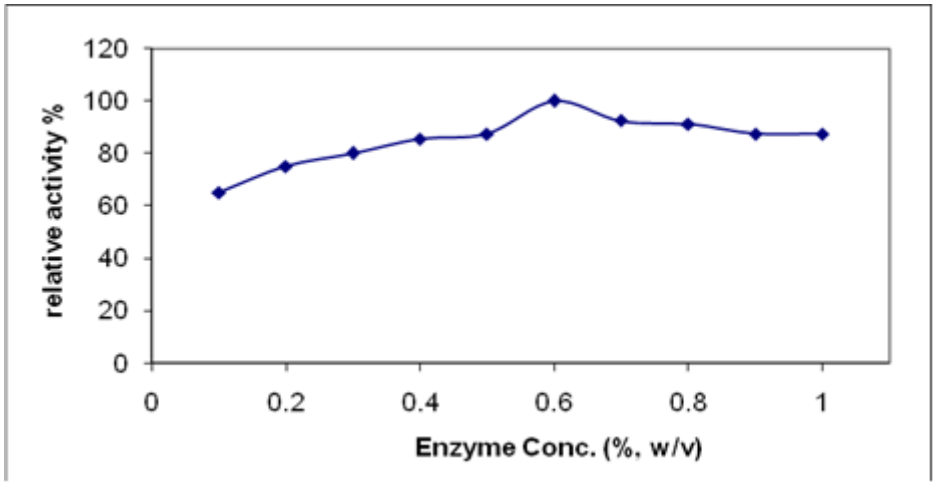


Fig (7): Effect of enzyme concentrations on the reaction activity of crude papainenzyme.

Table 8 . Effect of enzyme concentration on the reaction activity of crude papaya pectinesterase (PE) enzyme**:-**

Enzyme conc. (ml)	Activity (units/ml)	Specific activity U/mg protein	Relative activity (%)
1	**1.2**	**2.5**	**8.82**
2	**2**	**4.16**	**14.68**
3	**2.8**	**5.83**	**20.57**
4	**4**	**8.33**	**29.40**
5	**5.2**	**10.83**	**38.23**
6	**6.4**	**13.33**	**47.05**
7	**7.6**	**15.83**	**55.88**
8	**9.2**	**19.16**	**67.63**
9	**10**	**20.83**	**73.53**
10	**11.2**	**23.33**	**82.35**
11	**12.4**	**25.83**	**91.18**
12	**13.6**	**28.33**	**100.00**
13	**13.6**	**28.33**	**100.00**
14	**13.2**	**27.5**	**97.07**
15	**13.2**	**27.5**	**97.07**

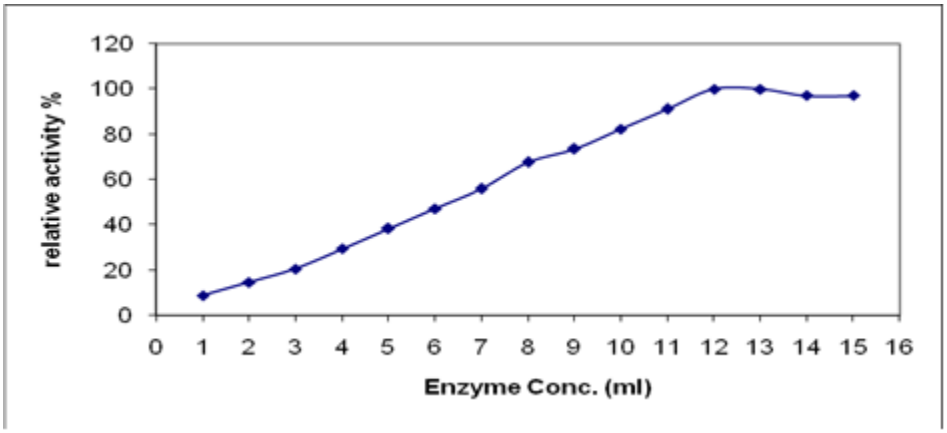


Fig (8): Effect of enzyme concentrations on the reaction activity of crude papaya pectinesterase (PE) enzyme.

Kinetic parameters of crude papain and papaya pectinesterase (PE) enzymes:-

The effect of substrate concentrations on the reaction velocity of crude papain enzyme was evaluated. The reaction mixture was carried out at different concentrations, i.e. (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4 and 1.5% (w/v) of casein as a substrate. The obtained results are illustrated in Table (9) and Fig. (9a, b). By plotting the obtained activity against the concentration of the substrate, the curve was obtained, Fig. (9a).From these results, the Michaelis-Menten constant and maximal reaction rate of the crude papain were determined and equalled to K_m = 0.43 g/100 mlphosphate buffer solution and V_{max} = 13.68units/ml /min, respectively. The acquired K_m by **Lineweaver and Burk** plots (Fig 9c), was equally to that obtained by experimentally curve. These values are higher than that reported by **Shaw *et al.* (1987)** who found that kinetic parameters were calculated as 0.12 g/100 ml for K_m and 8.90units/ml/min for V_{max} in the case of soluble papain enzyme. However, the activity of the crude papaya pectinesterase as a function of pectin concentration was also investigated. The reaction was carried out at different concentrations, i.e. 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0 mg/ml of pectin as a substrate. The obtained results were illustrated in Table (10) and Fig (10a,b). From these results, the Michaelis-Menten constant and maximal reaction rate of the crude papaya pectinesterase were determined and equaled to K_m = 3.2mg/ml buffer solution and V_{max} = 15.6units/ml, respectively.

The obtained K_m by **Lineweaver and Burk**plotsFig. (10c) was equally to that obtained by experimentally curve. These values are different from that reported by **Maria *et al.* (2007)** who found that kinetic parameters were calculated as 2.84 mg/ml for K_m and 64.10 units/mg proteins for V_{max} in the case of pectinesterase enzyme from hawthorn fruits.

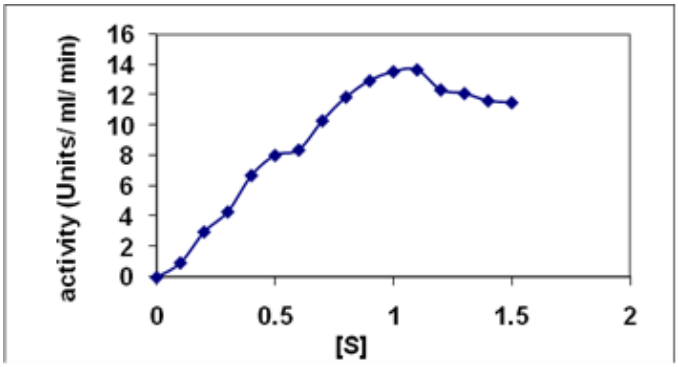
The present variation between the obtained K_m and V_{max} results of papain and pectinesterase may be due to that there were different concentrations of the both enzymes in one ml of the different experiments which related to the different sources of papain or pectinesterase extracted or obtained.

From the obtained results, it can conclude that papaya fruits contain benefits industrial enzymes such as papain and pectinesterase. Therefore the research is concentrated on the optimum conditions (PH, temperature, enzyme concentration, substrate concentration) about the production and the activity of both enzymes to achieve ideal uses of them.

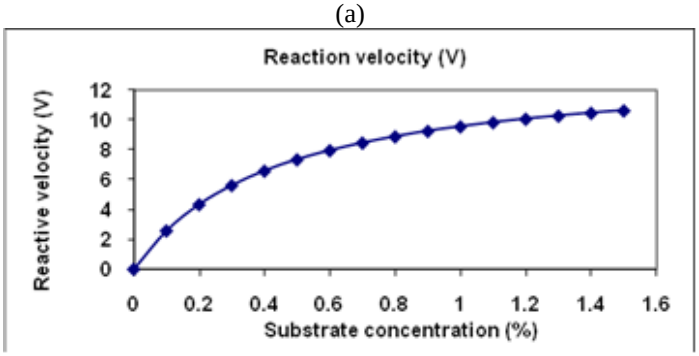
Table 9.Effect of substrate concentrations on the reaction velocity of crude papain enzyme.

Substrate concentration (%)	[1/S]	Activity Units/ml	Reaction velocity (V)*	[1/V] $\times 10^{-1}$
0.1	**10.00**	**0.96**	**2.58**	**3.87**
0.2	**5.00**	**3.00**	**4.34**	**2.30**
0.3	**3.33**	**4.32**	**5.62**	**1.78**
0.4	**2.50**	**6.72**	**6.59**	**1.52**
0.5	**2.00**	**8.04**	**7.35**	**1.36**
0.6	**1.66**	**8.40**	**7.96**	**1.26**
0.7	**1.43**	**10.32**	**8.47**	**1.18**
0.8	**1.25**	**11.88**	**8.89**	**1.12**
0.9	**1.11**	**12.96**	**9.26**	**1.07**
1.0	**1.00**	**13.56**	**9.56**	**1.04**
1.1	**0.91**	**13.68**	**9.84**	**1.01**
1.2	**0.83**	**12.36**	**10.07**	**0.99**
1.3	**0.77**	**12.12**	**10.28**	**0.97**
1.4	**0.71**	**11.64**	**10.47**	**0.95**
1.5	**0.66**	**11.52**	**10.63**	**0.94**

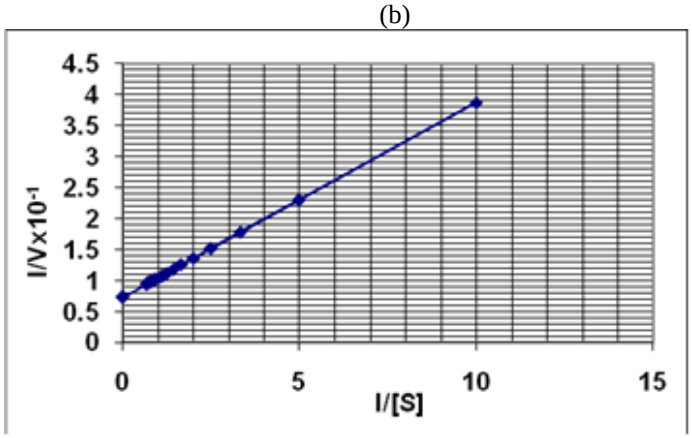
V_{max}= 13.68 Units/ml. **K_m = 0.43 g/100 ml** * $V = \frac{V_{max} \times [S]}{K_m + [S]}$



(a)



(b)



(c)

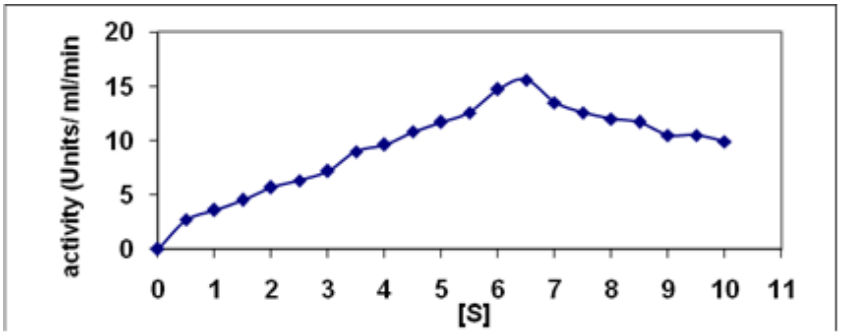
Fig (9): Effect of substrate concentrations on the reaction velocity of crude papain enzyme.

Table 10. Effect of substrate concentrations on the reactionvelocity of crude papaya pectinesterase (PE)enzyme.

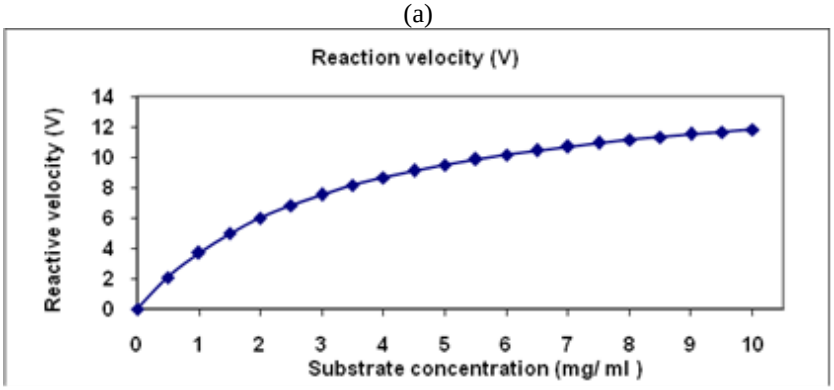
Substrate concentration (mg/ml)	[1/S]	Activity Units/ml	Reaction velocity (V)*	[1/V] $\times 10^{-1}$
0.5	**2.00**	**2.7**	**2.11**	**4.73**
1.0	**1.00**	**3.6**	**3.71**	**2.69**
1.5	**0.66**	**4.5**	**4.98**	**2.00**
2.0	**0.50**	**5.7**	**6.00**	**1.66**
2.5	**0.40**	**6.3**	**6.84**	**1.46**
3.0	**0.33**	**7.2**	**7.55**	**1.32**
3.5	**0.28**	**9.0**	**8.15**	**1.22**
4.0	**0.25**	**9.6**	**8.66**	**1.15**
4.5	**0.22**	**10.8**	**9.12**	**1.09**
5.0	**0.20**	**11.7**	**9.51**	**1.05**
5.5	**0.18**	**12.6**	**9.86**	**1.01**
6.0	**0.166**	**14.7**	**10.17**	**0.98**
6.5	**0.153**	**15.6**	**10.45**	**0.95**
7.0	**0.142**	**13.5**	**10.71**	**0.93**
7.5	**0.133**	**12.6**	**10.93**	**0.91**
8.0	**0.125**	**12.0**	**11.14**	**0.89**

8.5	**0.117**	**11.7**	**11.33**	**0.88**
9.0	**0.111**	**10.5**	**11.51**	**0.86**
9.5	**0.105**	**10.5**	**11.66**	**0.85**
10	**0.100**	**9.9**	**11.82**	**0.84**

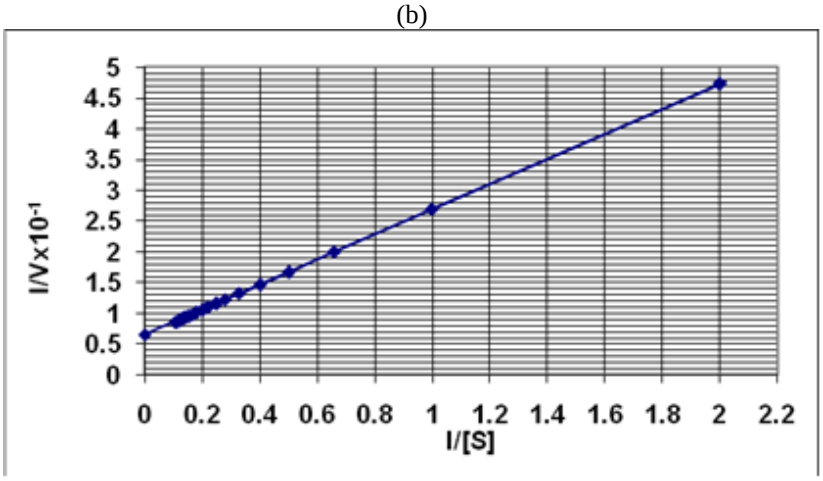
V_{max}= 15.6 Units/ml. **K_m = 3.2 mg/ml** * $V = \frac{V_{max} \times [S]}{K_m + [S]}$



(a)



(b)



(c)

Fig. (10): Effect of substrate concentrations on the reaction velocity of crude papaya pectinesterase (PE) enzyme.

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إنتاج وتقييم إنزيم البابايين والبكتين إستيريز من ثمار الباباظ

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تهدف هذه الدراسة الى أنتاج بعض الأنزيمات مثل انزيم البابايين وانزيم البكتين استيريز من ثمار الباباظ ودراسة افضل الظروف للحصول على اعلى معدل استخلاص لهم.

دراسة العوامل التى تؤثر على سرعة التفاعل والنشاط لكلا من انزيم البابيين والبكتين استيريز من تركيز القوة الأيونية لNaCl , PH, درجة الحرارة , تركيز الأنزيم, تركيز مادة التفاعل المستخدمة وايجاد كلا من ثابت ميكاليسK_m والسرعة القصوى V_{max} لامكانية التوصل الى انسب الظروف المثلي لنشاط هذه الانزيمات.

اوضحت النتائج ان كمية العصارة اللبنية المستخلصة كانت 6 جم /كجم ثمار وبعد التجفيف كانت الكمية 1,5 جم /كجم ثمار حيث ان نسبة الرطوبة كانت 74,6% للعصارة اللبنية .

وجد ان افضل تركيز من كلوريد الصوديوم لاستخلاص انزيم البكتين استيريز كانت 1,5 مولر حيث وصل نشاط الإنزيم الى اعلى معدل لنشاط وهو 6,25 وحدة/ ملجم بروتين.

كذلك وجد ان درجة النشاط والمحتوى البروتينى لانزيم البابيين هى 7,68 وحدة /ملل و 0,74 ملجم/ ملل على التوالى. كذلك بالنسبة لانزيم البكتين استيريز كانت 2,8 وحدة /ملل و 0,48 ملجم / ملل على التوالى.

وجد ان افضل تركيز من كلوريد الصوديوم المطلوب لنشاط انزيم البكتين استيريز هو 0,3 مولر .

ان افضل درجة pH اللازم لنشاط كلا من البابايين والبكتين استيريز هو 6,4 و 7 على التوالى .

ان افضل درجة حرارة اللازمة لنشاط كلا من البابايين والبكتين استيريز هى 60 و55 درجة مئوية على التوالى .

ان افضل تركيز للانزيم اللازم لنشاط كلا من البابايين والبكتين استيريز هو 0,6جم/100ملل محلول منظم و 12 مل على التوالى .

ان افضل تركيز للمادة المتفاعلة اللازمة لنشاط كلا من البابايين والبكتين استيريز هو 1,1جم/100ملل محلول منظم و 6,5 ملجم/ملل.

كذلك وجد ان قيمة كلا من K_m , V_{max} لكلا من انزيم البابيين والبكتين استيريز هى 0,43 جم / 100مل محلول منظم , 13,68 وحدة/ ملل بالنسبة للبابايين و 3,2 جم/ 100ملل و 15,6 وحدة/ ملل بالنسبة للبكتين استيريز على التوالى .